

AMPLIFY VH GENES WITHOUT USING VH SEQUENCES

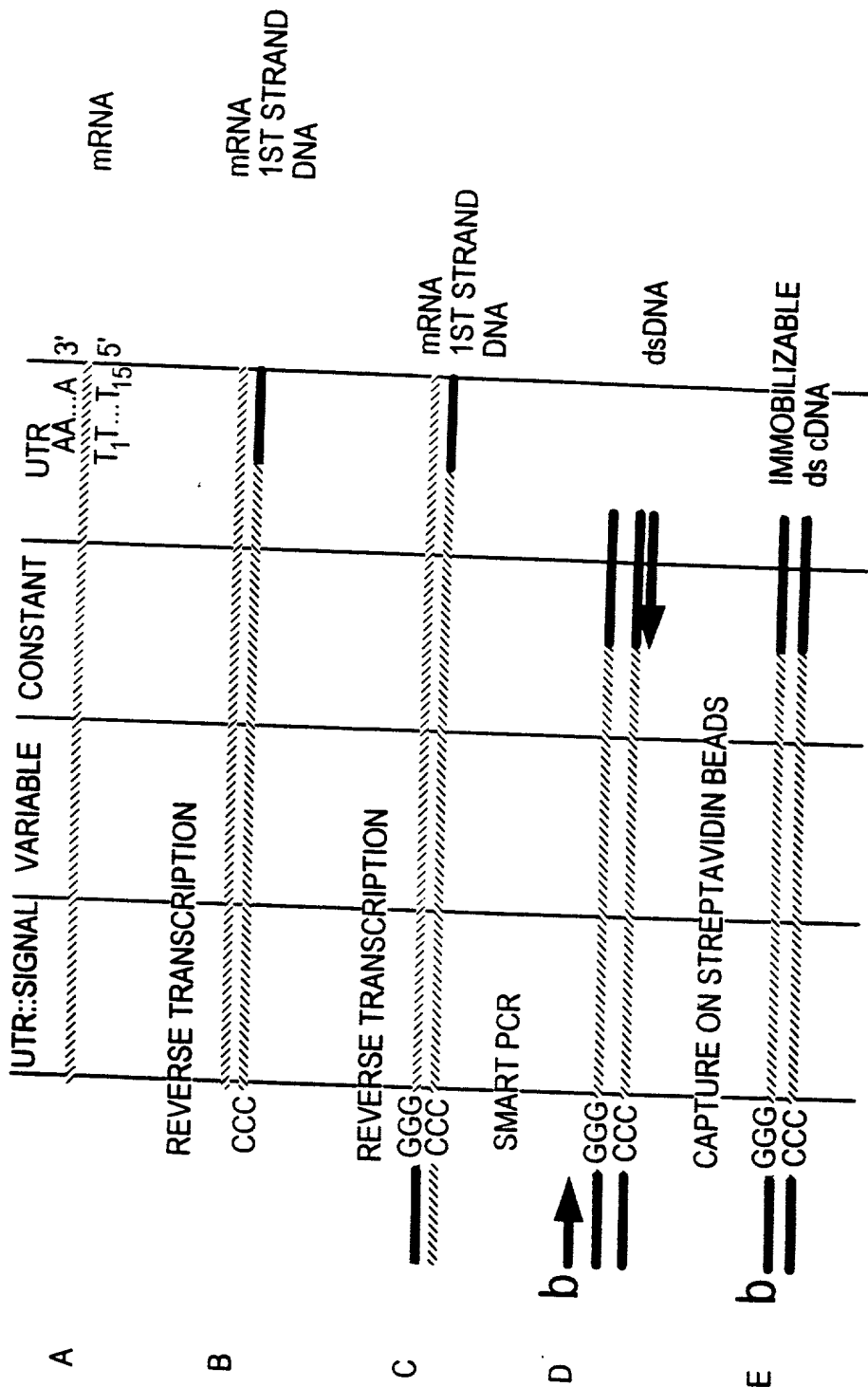


FIG. 1

AMPLIFY VL GENES WITHOUT USING VL SEQUENCES

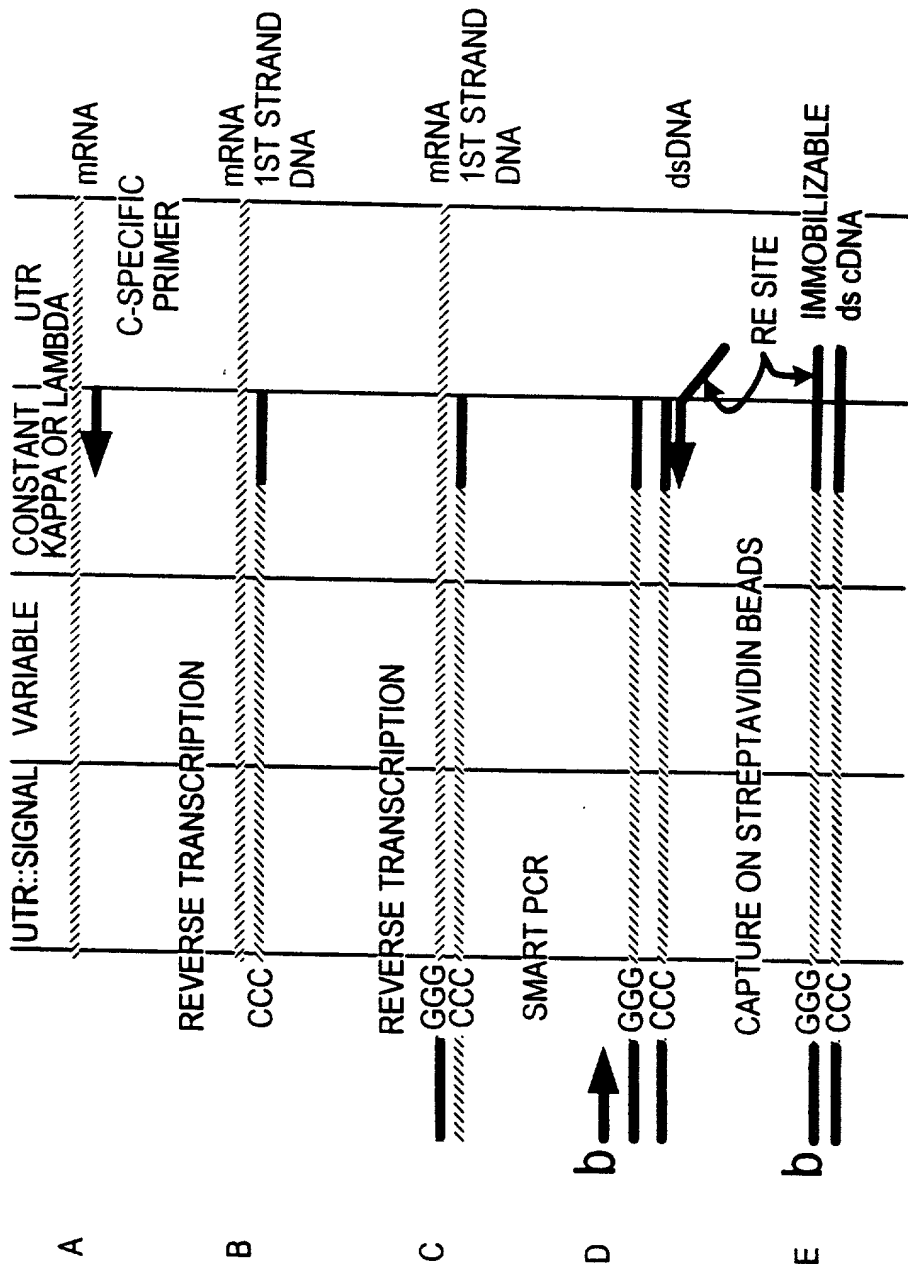


FIG. 2

RACE non-biased antibody V-gene amplification

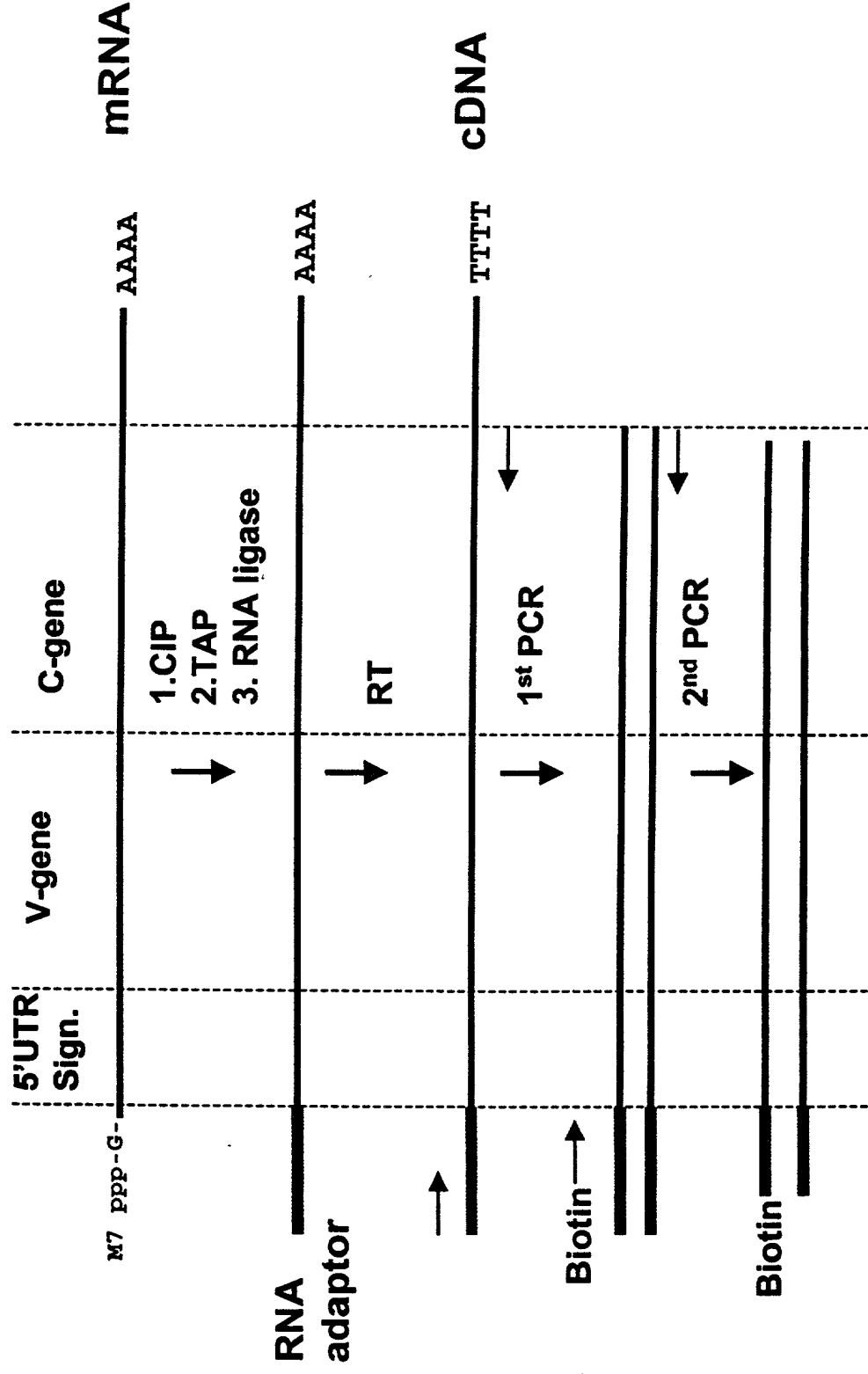


FIG. 3

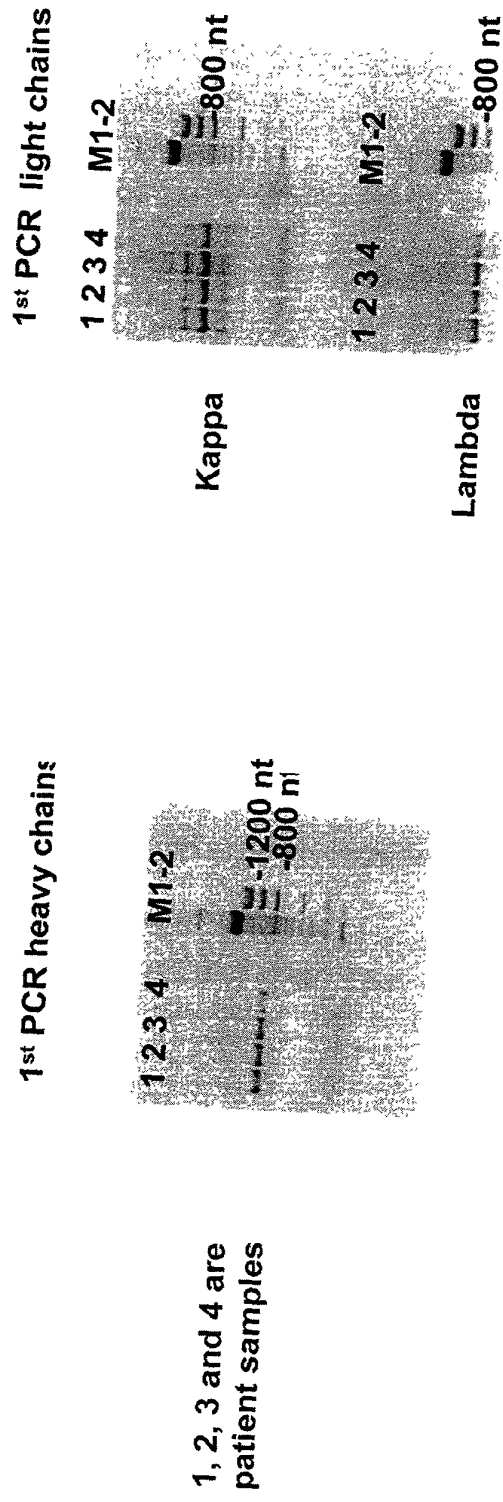
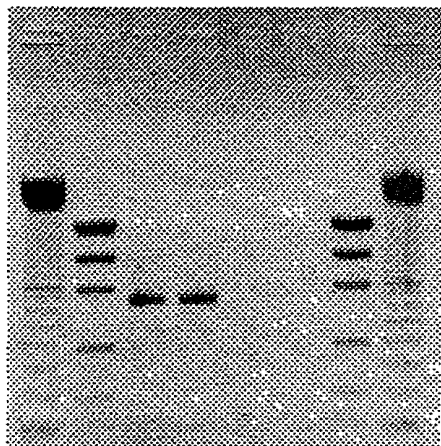


FIG. 4

1 2 3 4 5 6 7 8



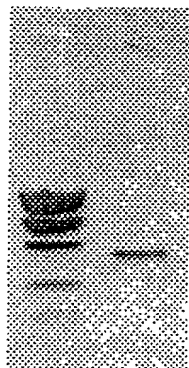
Gel analysis of PCR product from extender-kappa amplification

Approx. 75ng/5 μ l \rightarrow 15ng/ μ l

- 1 - 100bp
- 2 - LDM
- 3 - 50ng template
- 4 - 10ng template
- 5 - ssDNA unligated
- 6 - negative control
- 7 - LDM
- 8 - 100bp

FIG. 5

1 2



Gel purified PCR product from extender-kappa amplification

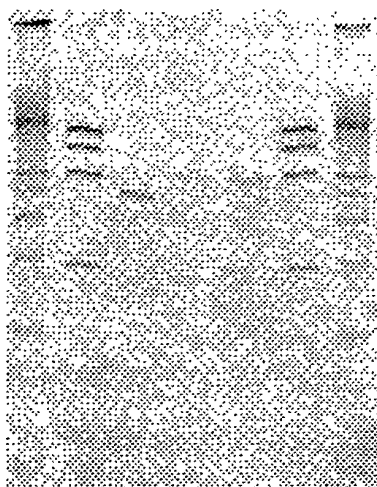
Concentration : $\pm 35\text{ng}/\mu\text{l}$

1 - LDM

2 - 1µl purif.

FIG. 6

1 2 3 4 5 6 7



Gel-analysis of digested κ -ssDNA

1 μ l digested ssDNA \approx 8ng ssDNA

Total volume of 50 μ l = 400ng ssDNA

→ 400ng ssDNA available for ligation of the bridge-extenders

1 - 100bp

2 - LDM

3 - 1 μ l ssDNA pure

4 - 4 μ l beads after dig.

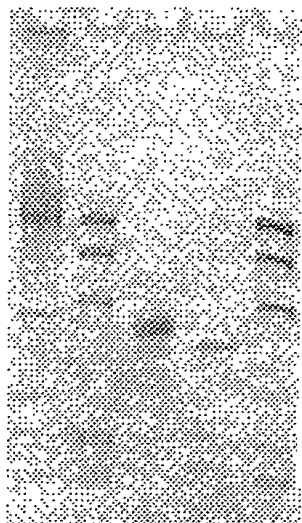
5 - 8 μ l beads after dig.

6 - LDM

7 - 100bp

FIG. 7

1 2 3 4 5



Gel analysis of extender – cleaved kappa ligation

20ng/5 μ l eluted material \rightarrow 4ng/ μ l

- 1- 100bp
- 2 - LDM
- 3 - Ligationmix, 4 μ l
- 4 - Unligated ssDNA
- 5 - LDM

FIG. 8

Cleavage and ligation Kappa light chains

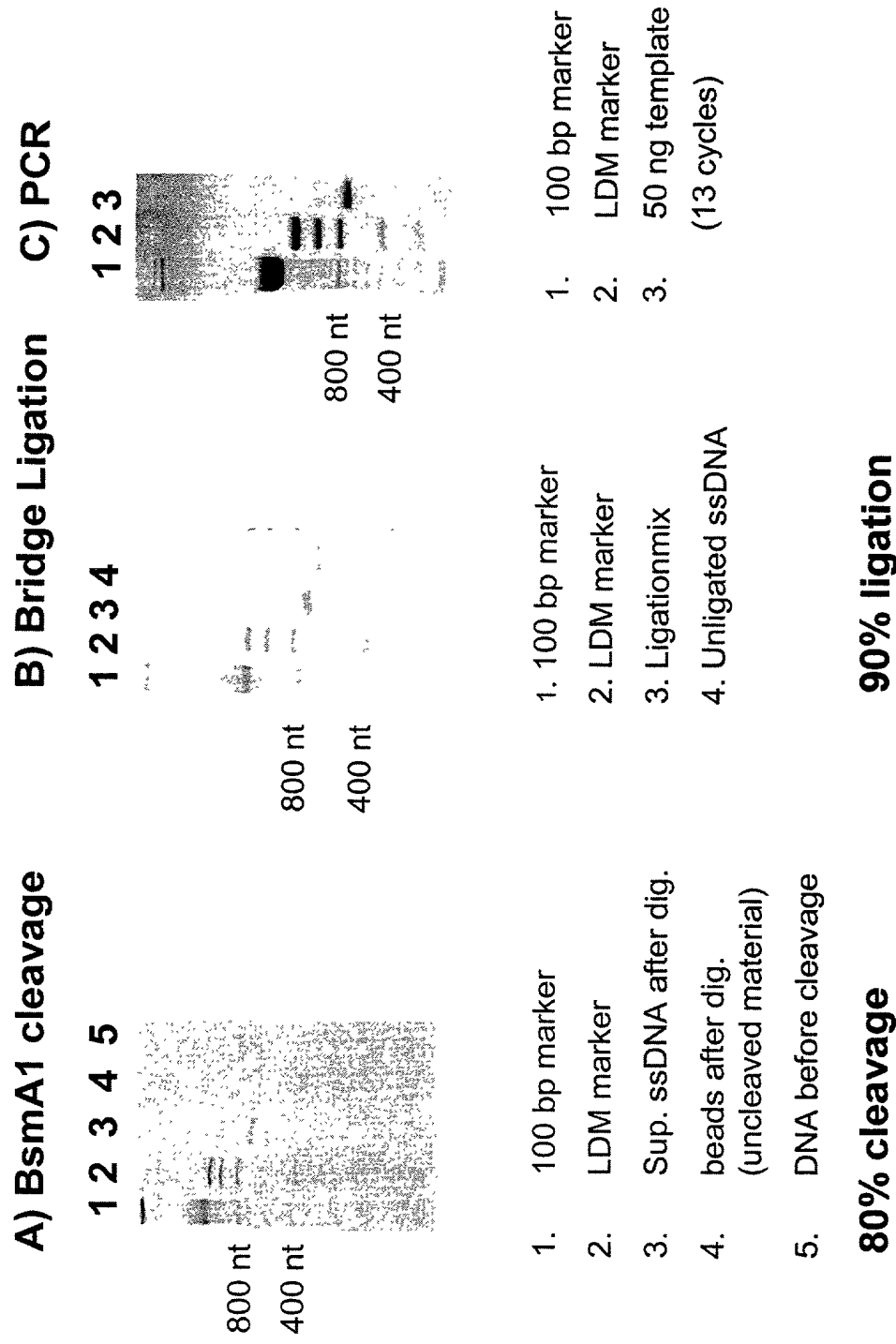


FIG. 9

VH-CDR1

1 Y 1 M 1

VH-CDR2

2 I 2 3 S G G 1 T 1 YADSVKG

FIG. 10

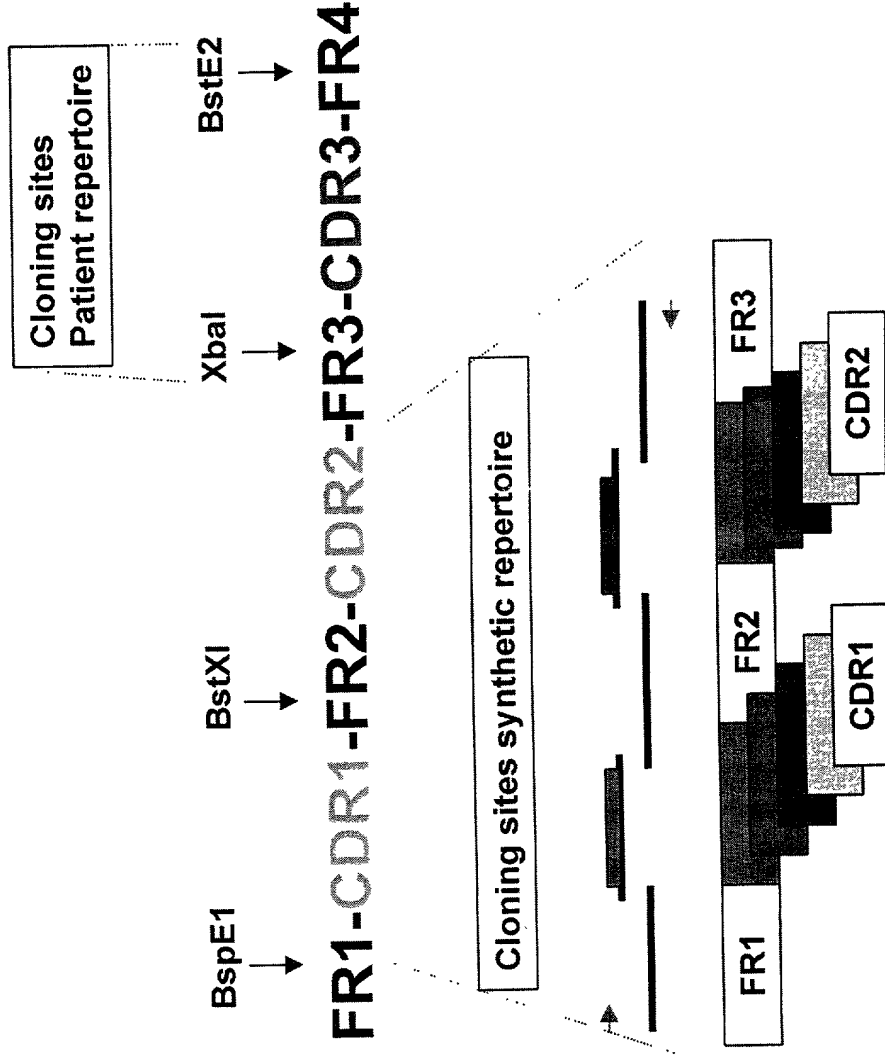


FIG. 11

Cleavage antibody light chain genes

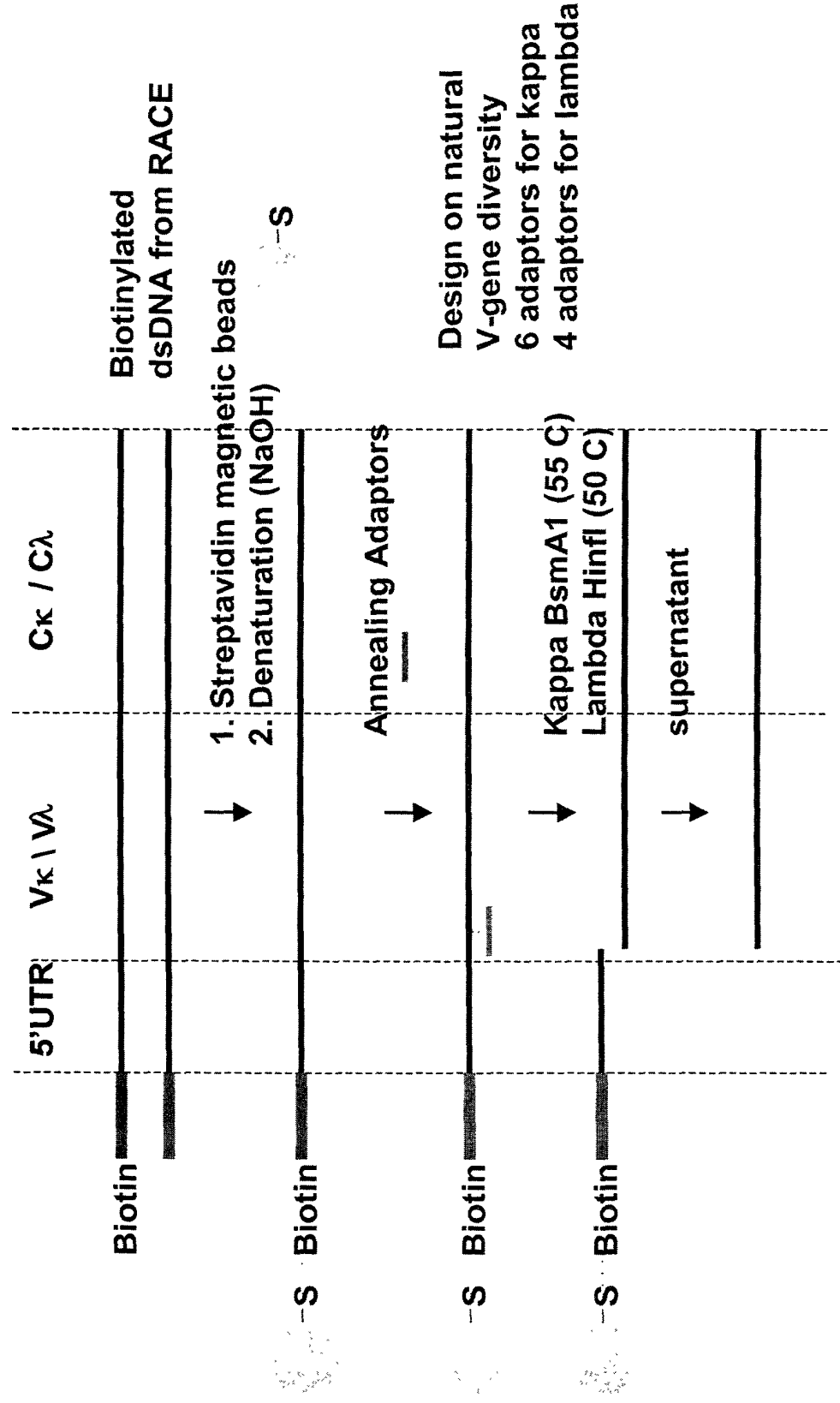


FIG. 12A

Ligation of cleaved light chains

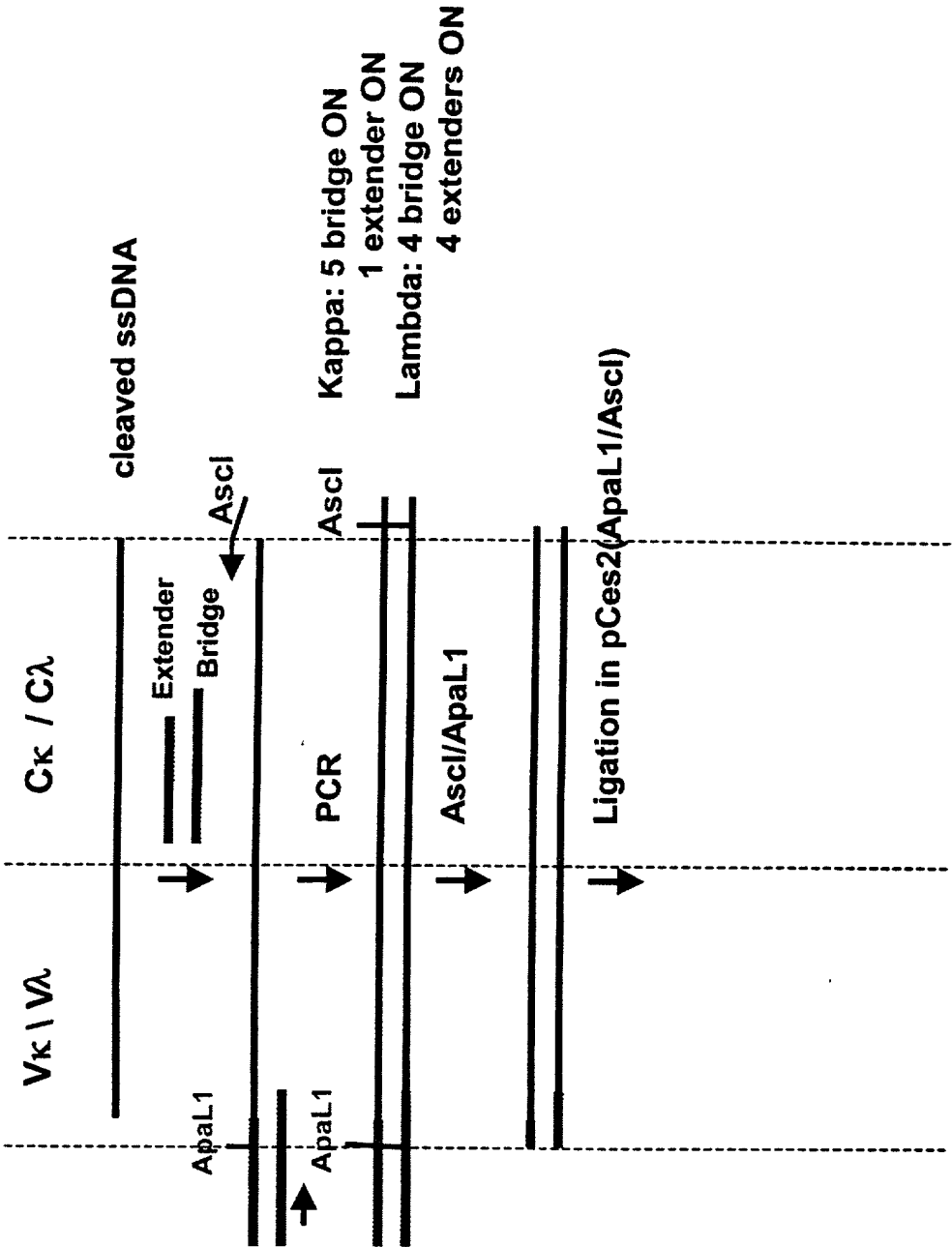


FIG. 12B

Figure 3: Cleavage and ligation lambda light chains

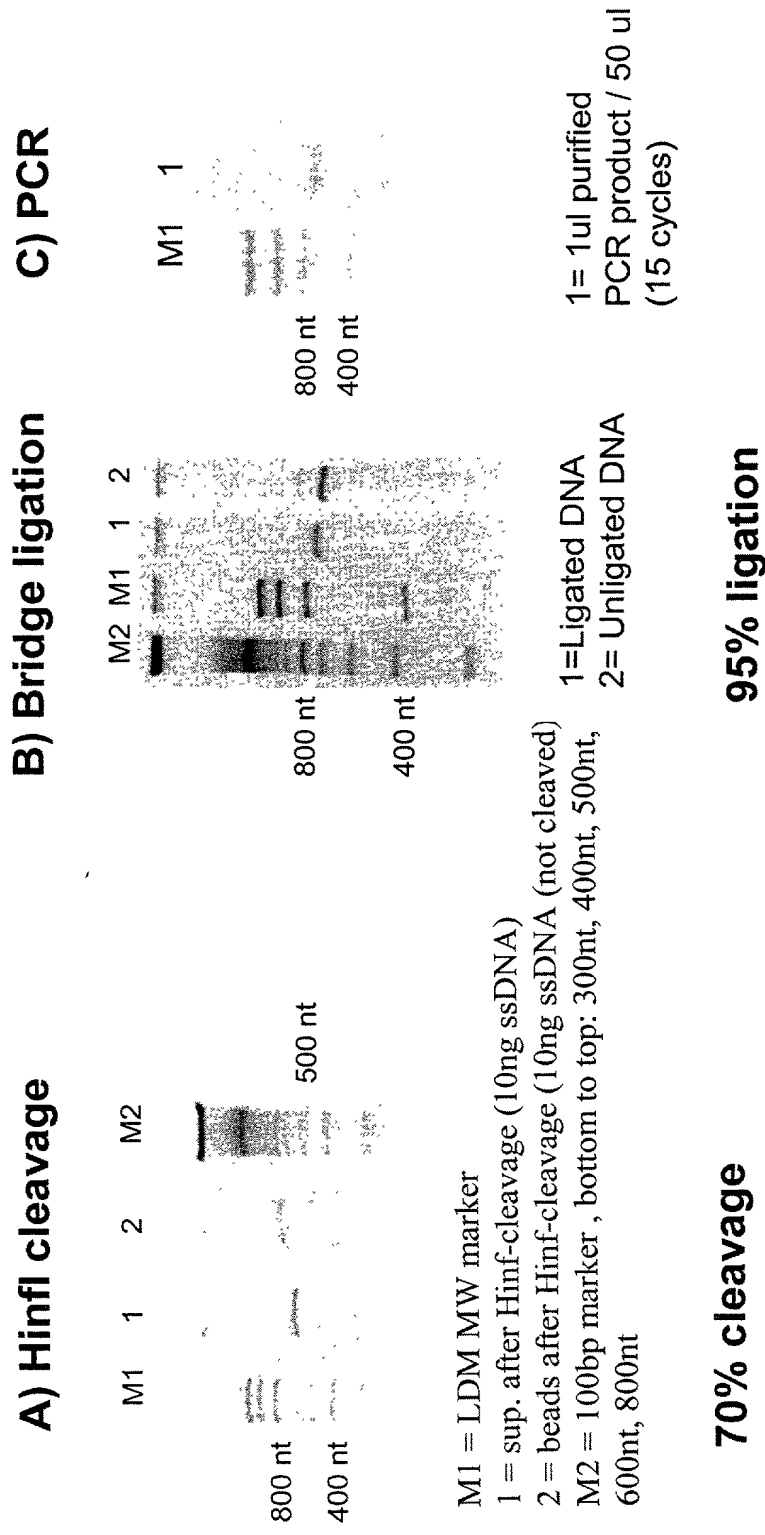


FIG. 13

Ligation heavy chain CDR3 diversity

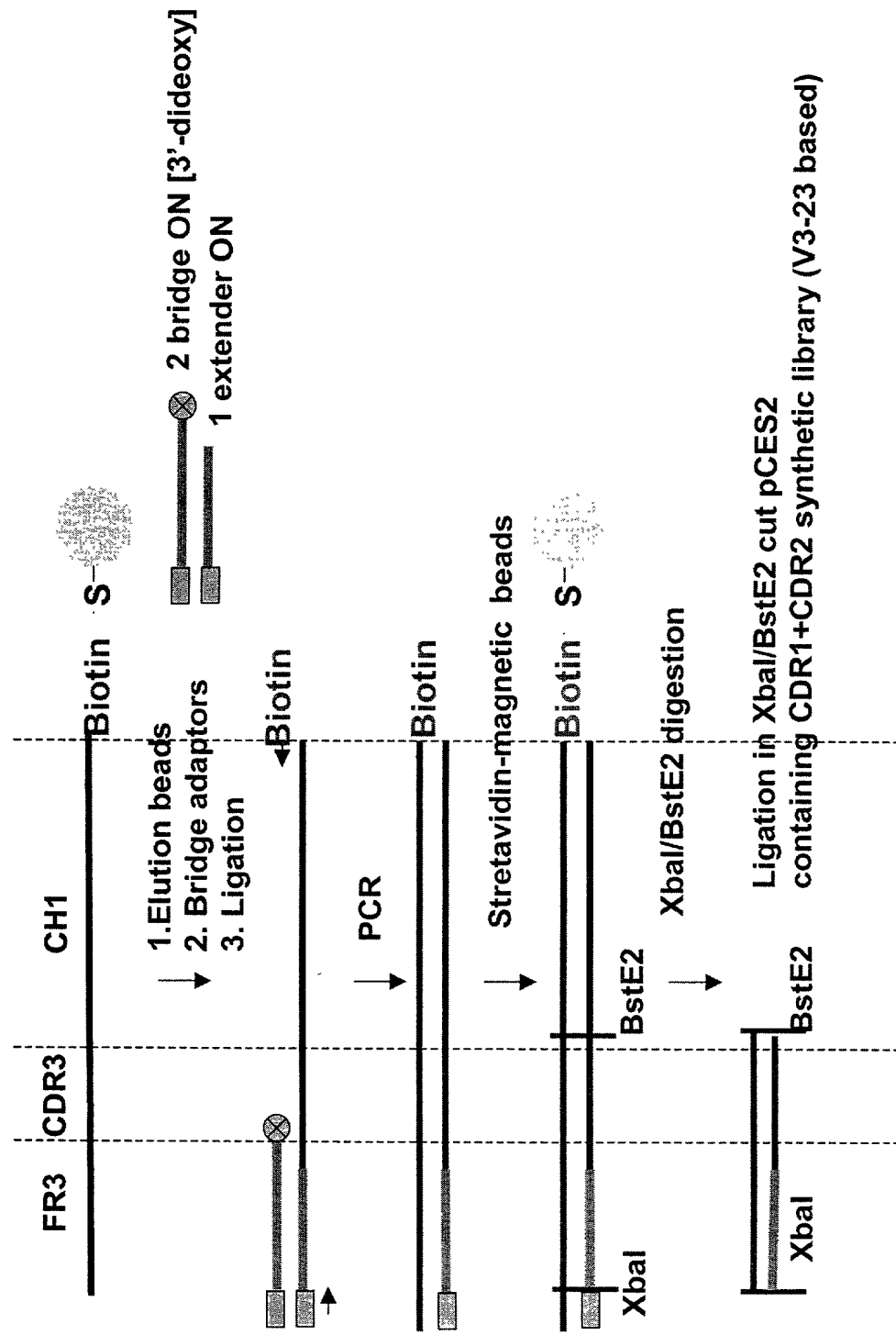
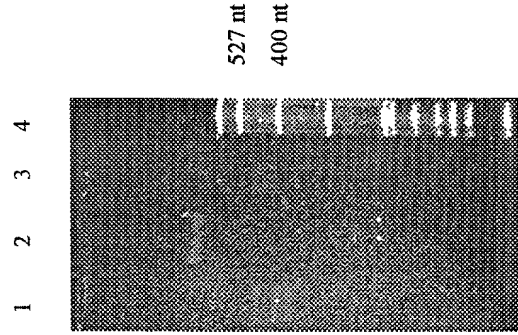


FIG. 14B

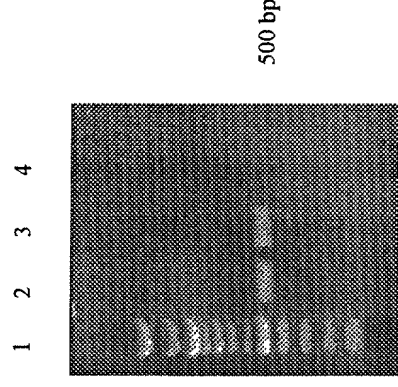
Cleavage and ligation Heavy Chain

A) HpyCH4III cleavage



1 = Cleaved DNA eluted from PN column
2 = Beads after HpyCH4III digestion
3 = Supernatant after cleavage
4 = MspI digest of pBR322

B) PCR



1 = NEB 100bp ladder
2 = 5ul/100ul PCR product 20 cycles; sample A
3 = 5ul/100ul PCR product 20 cycles; sample B
4 = no template

FIG. 15

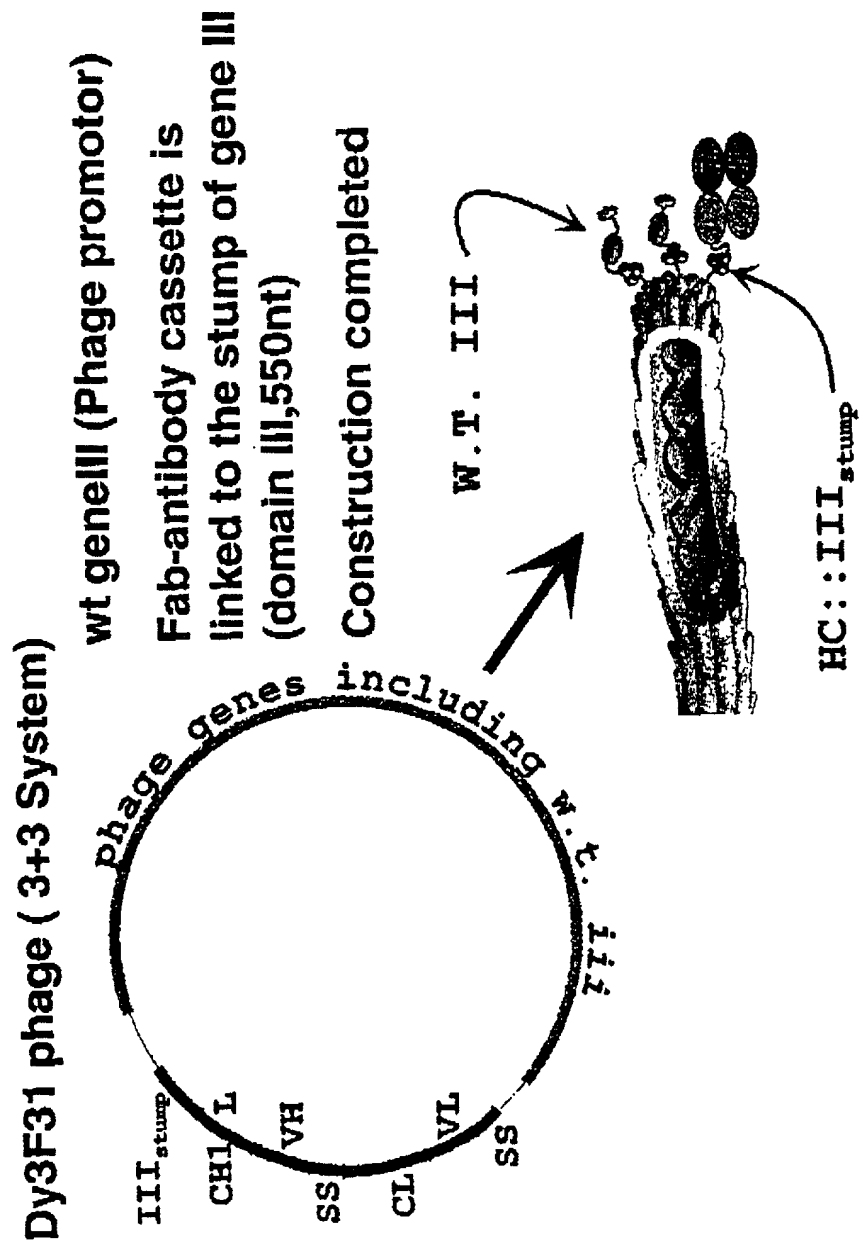


FIG. 16

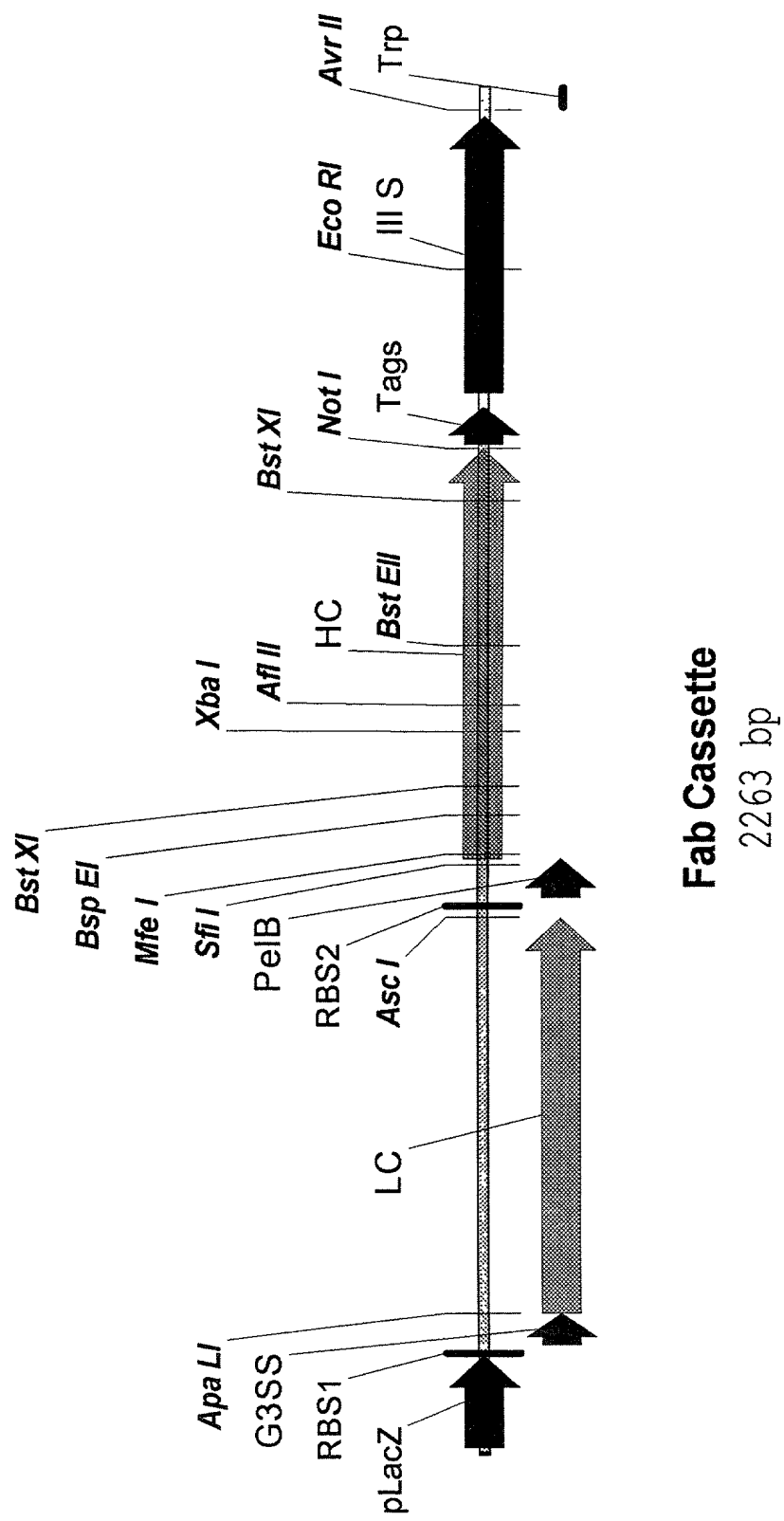


FIG. 17

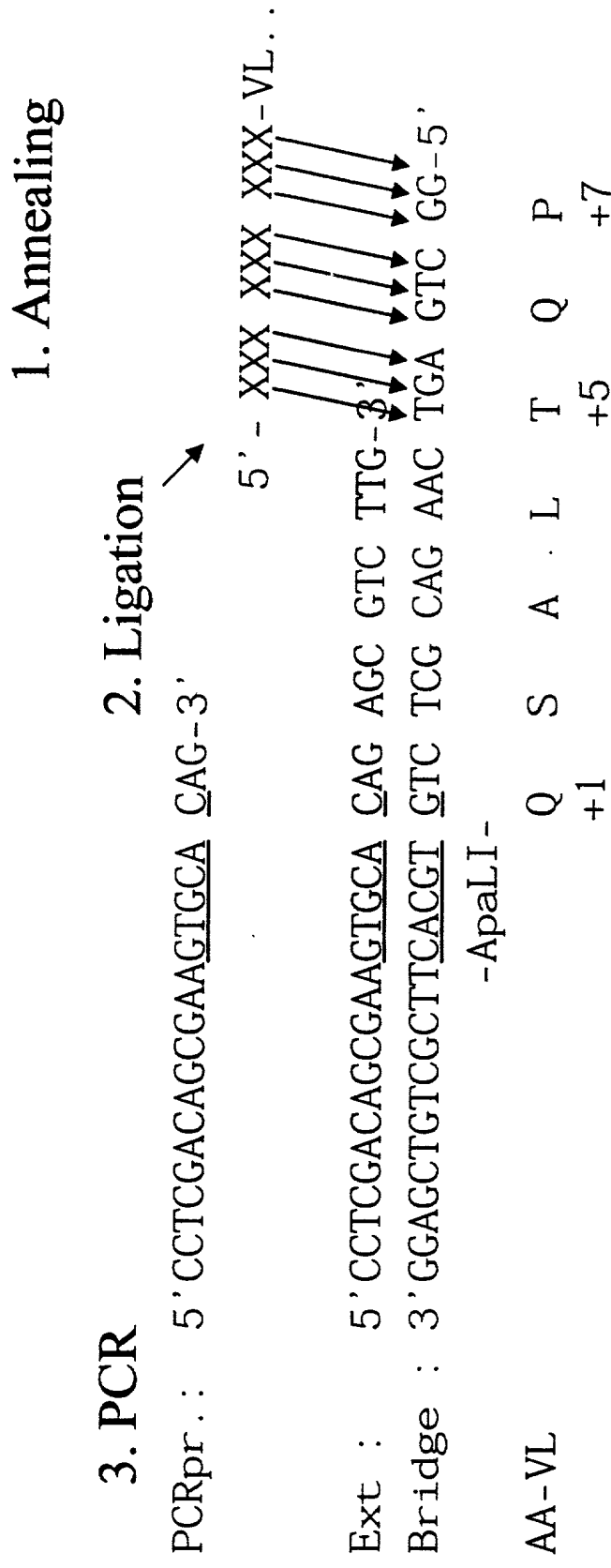


FIG. 18

3. PCR

PCRpr.: 5'-CCTCTCTGCACA GTGCA CAA GAC-3'

1. Annealing

5'-XXX-XXX X-VL..

2. Ligation

Ext : 5'-CCTCTCTGCACA GTGCA CAA GAC ATC CAG ATG ACC CAG TCT CC
 Bri : 3'-GG ..
 AA-VL ..
 -ApaLI-
 Q D I Q M T Q S P S S
 +1 +8 +9 +10
 GGT AGG AGG G-5'

FIG. 19

3. PCR

PCRpr.:

5' -GAC TGG GTG TAG TGA TCT AG-3'

+70

(FR3)

$$\begin{matrix} * \\ * \\ \geq \end{matrix}$$
R
S

SN

Y

C

+92

1. Annealing

Bridge : 5' -G GTG TAG TGA TCT AGT GAC AAC TCT ... TAC TAT TGT GCG AAA-3'

Ext : 3' -C CAC ATC ACT AGA TCT CTG TTG AGA ... ATG ATA-5'

-XbaI-

2. Ligation

3'-XXX XXX-VH

FIG. 20